Dehydration of Bile Acids and their Derivatives. X. A Study of the Physical Properties of Various 3α-Hydroxycholenates and their Derivatives, with Special Reference to their Optical Rotatory Dispersions

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In the previous papers[1,2], the synthesis of methyl 3α-hydroxy-$\Delta^{8(14)}$-cholenate and 3α-hydroxy-Δ^{14}-cholenate was reported. Besides these unsaturated bile acids, various 3α-hydroxycholenic acids of known structure have already been reported. The present paper deals with the study of the correlation of the physical properties with the positions of the double bonds of these 3α-hydroxycholenates and their derivatives, above all, the optical rotatory dispersions of these bile acids are discussed in detail because Djerassi[3] has shown that the rotatory dispersion curve (the RD curve) is an excellent tool for elucidating the structure of steroids.

The ultraviolet absorption spectra of the unsaturated bile acids of various types were studied; their ε values are shown in Table I. The bile acids with exocyclic, tetrasubstituted double bonds ($\Delta^{8(14)}$) give much higher ε values than do unsaturated bile acids of the other types. On the other hand, bile acids with a disubstituted ethylenic linkage, such as 3-keto-Δ^{6}-cholenate, give much lower ε values than those with trisubstituted (Δ^{7}, $\Delta^{9(11)}$, Δ^{14}) or endocyclic, tetrasubstituted (Δ^{8}) double bonds. These results agree fairly well with those reported by Bladon et al.[4]

As Barton[5] has reported, the position of a double bond in the steroid molecule can be deduced by calculating the molecular rotation increment due to the introduction of the double bond. As a supplement to the data in this field, the molecular rotations of various available unsaturated and corresponding saturated bile acids were measured; the calculated M_D contributions of the double bonds are summarized in Table II. It was an interesting finding that the positive sign of the M_D contributions due to the double bonds in ring B or C is strongly inverted when the C_{12}-carbonyl group is simultaneously introduced; e. g., the

TABLE I. ULTRAVIOLET ABSORPTION IN ETHANOL

Unsaturated bile acid	λ_{max}	ε_{max}	ε_{210}	ε_{215}	ε_{220}
Methyl 3α-hydroxy-Δ^{7}-cholenate (I)	210	3000	3000	1930	580
Methyl 3α-acetoxy-Δ^{7}-cholenate (II)	210	2290	2290	1390	650
Methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (III)	214	5900	5040	5560	3470
Methyl 3α-hydroxy-Δ^{14}-cholenate (IV)	209	2490	2410	1020	370
Methyl 3-keto-Δ^{4}-cholenate (V)	240	14129	2627	4606	6174
Methyl 3-keto-$\Delta^{7,9(11)}$-choladienate (VI)	245	10920	3030	4190	5610
Methyl 3-keto-Δ^{8}-cholenate (VII)	211	2420	2390	1760	1240
Methyl 3-keto-$\Delta^{8(14)}$-cholenate (VIII)	214	5190	4000	5030	4050
Methyl 3-keto-$\Delta^{9(11)}$-cholenate (IX)	211	2200	2080	1480	880
Methyl 3-keto-Δ^{14}-cholenate (X)	210	1990	1990	980	290
Methyl 3-keto-Δ^{7}-cholenate (XI)	212	2320	2230	2190	1620
Methyl 3-keto-Δ^{6}-cholenate (XII)	214	320	140	300	180
Methyl 3α-acetoxy-12-keto-$\Delta^{9(11)}$-cholenate (XIII)	239	10300	2250	3860	5300
Methyl 3α-acetoxy-12-keto-Δ^{8}-cholenate (XIV)	213	3980	3100	3450	2420
Methyl 3α-acetoxy-12-keto-$\Delta^{8(14)}$-cholenate (XV)	215	5270	3500	5270	4790
Methyl 3, 12-diketo-$\Delta^{8(14)}$-cholenate (XVI)	215	5940	4500	5940	4990
Methyl 3, 12-diketo-Δ^{14}-cholenate (XVII)	210	2140	2140	1090	280

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[1] R. Osawa and K. Yamasaki, This Bulletin, **32**, 1302 (1959).

[2] F. Nakada, R. Osawa and K. Yamasaki, ibid., **34**, 538 (1961).

[3] C. Djerassi, " Optical Rotatory Dispersion ", McGraw-Hill Book Co., Inc., New York (1960).

[4] P. Bladon, H. B. Henbest and G. W. Wood, *J. Chem. Soc.*, **1952**, 2737.

[5] D. H. R. Barton, ibid., **1946**, 1116.

TABLE II. MOLECULAR ROTATION DIFFERENCE

Unsaturated bile acid	$(M)_D$ unsaturated	$(M)_D$ saturated	M_D contributions of double bonds
Methyl 3α-hydroxy-Δ^{7}-cholenate (I)	+207	+ 90	+117
Methyl 3α-acetoxy-Δ^{7}-cholenate (II)	+370	+194	+176
Methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (III)	+241	+194	+ 47
Methyl 3α-hydroxy-Δ^{14}-cholenate (IV)	+213	+ 90	+113
Methyl 3-keto-Δ^{4}-cholenate (V)	+235	+124	+111
Methyl 3-keto-$\Delta^{7,9(11)}$-choladienate (VI)	+338	+124	+214
Methyl 3-keto-Δ^{8}-cholenate (VII)	+ 85	+124	− 39
Methyl 3-keto-$\Delta^{8(14)}$-cholenate (VIII)	+263	+124	+139
Methyl 3-keto-$\Delta^{9(11)}$-cholenate (IX)	+127	+124	+ 3
Methyl 3-keto-Δ^{14}-cholenate (X)	+146	+124	+ 22
Methyl 3-keto-Δ^{7}-cholenate (XI)	+204	+124	+ 80
Methyl 3-keto-Δ^{6}-cholenate (XII)	−201	+124	− 77
Methyl 3α-acetoxy-12-keto-$\Delta^{9(11)}$-cholenate (XIII)	+489	+692	−203
Methyl 3α-acetoxy-12-keto-Δ^{8}-cholenate (XIV)	+613	+692	− 79
Methyl 3α-acetoxy-12-keto-$\Delta^{8(14)}$-cholenate (XV)	+244	+692	−448
Methyl 3, 12-diketo-$\Delta^{8(14)}$-cholenate (XVI)	+ 56	+370	−314
Methyl 3, 12-diketo-Δ^{14}-cholenate (XVII)	+208	+370	−168

TABLE III. INFRARED FREQUENCIES FOR BILE ACIDS CONTAINING NUCLEAR DOUBLE BONDS OF VARIOUS TYPES (IN NUJOL)

Unsaturated bile acid	3000~3100 cm^{-1} (3.3μ)	1620~1700 cm^{-1} (6μ)	790~850 cm^{-1} (12μ)
Methyl 3α-hydroxy-Δ^{7}-cholenate	3018 (sh)*	1640 (st)*	745 (m)*
Methyl 3α-acetoxy-Δ^{7}-cholenate	3015 (sh)	1678 (sh)	808 (st)
Methyl 3-keto-Δ^{7}-cholenate	3055 (w) 3012 (sh)	1655 (sh)	795 (w)
Methyl 3-keto-Δ^{8}-cholenate	3018 (sh)	—	815 (w)
Methyl 3α-acetoxy-Δ^{8}-cholenate	3045 (sh)	—	840 (w)
Methyl 3-keto-$\Delta^{8(14)}$-cholenate	—	1645 (sh)	770 (w)
Methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate	—	—	840 (w)
Methyl 3α-hydroxy-Δ^{14}-cholenate	3055 (st)	1648 (m)	793 (m)
Methyl 3α-acetoxy-Δ^{14}-cholenate	3030 (m)	—	770 (m)
Methyl 3-keto-Δ^{14}-cholenate	3055 (m)	1648 (sh)	777 (st)
Methyl 3α-acetoxy-Δ^{5}-cholenate	—	1680 (sh)	800 (m) 840 (m)
Methyl 3α-hydroxy-Δ^{6}-cholenate	3040 (m)	—	740 (st)
Methyl 3-keto-Δ^{6}-cholenate	3040 (m)	1644 (sh)	740 (st)
Methyl 3α-acetoxy-$\Delta^{9(11)}$-cholenate	3020 (sh)	—	775 (w)
Methyl 3-keto-$\Delta^{9(11)}$-cholenate	3018 (sh)	—	825 (w)
Methyl 3α-hydroxy-Δ^{4}-cholenate	—	1660 (m)	812 (m)
Methyl 3-keto-Δ^{4}-cholenate	—	1620 (st)	800 (st)
Methyl 3-keto-$\Delta^{7,9(11)}$-cholenate	3020 (sh)	1605 (w)	800 (st)

* sh = shoulder st = strong
w = weak m = moderate

M_D contribution due to the $\Delta^{8(14)}$-bond of methyl 3α-acetoxy-12-keto-$\Delta^{8(14)}$-cholenate is −448, while the corresponding value of 3α-acetoxy-$\Delta^{8(14)}$-cholenate is +47.

As for the infrared absorption spectra of these unsaturated bile acids (Table III), the trisubstituted ethylenic derivatives (Δ^{4}, Δ^{5}, Δ^{7} and Δ^{14}) give more or less characteristic absorptions in the regions of 3.3, 6 and 10~15 μ, as Bladon et al.[7] have pointed out (the $\Delta^{9(11)}$-compounds are exceptions[6]).

While Djerassi et al.[8] have studied the correlation of the isolated ketonic functions in the saturated keto bile acids with the RD curves, the RD curves of unsaturated keto

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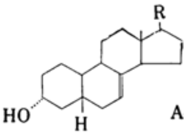
[6] R. N. Jones, P. Humphries, E. Packard and K. Dobriner, *J. Am. Chem. Soc.*, **72**, 86 (1950).

[7] P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch and G. W. Wood, *J. Chem. Soc.*, **1951**, 2402.

[8] C. Djerassi and W. Closson, *J. Am. Chem. Soc.*, **78**, 3761, (1956).



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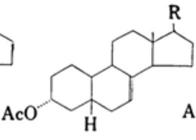

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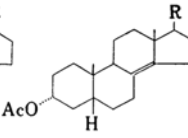

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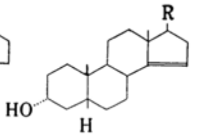

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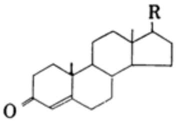

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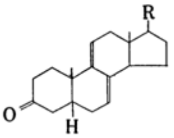

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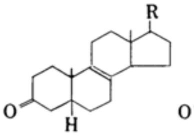

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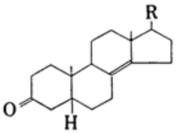

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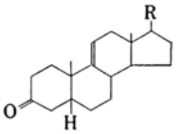

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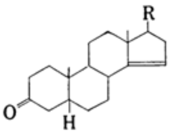

X

$R = -CH(CH_3)CH_2CH_2COOCH_3$

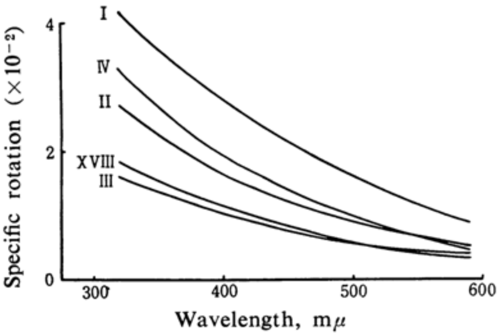

Fig. 1. Rotatory dispersion curves of: methyl 3α-hydroxy-Δ^{7}-cholenate (I), methyl 3α-acetoxy-Δ^{7}-cholenate (II), methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (III), methyl 3α-hydroxy-Δ^{14}-cholenate (IV) and methyl cholate (XVIII).

bile acids have not yet been covered in the literature. Following Djerassi's report⁹⁾, the background rotations of some unsaturated bile acids were measured and compared with that of a saturated bile acid, cholic acid (as methyl ester). As shown in Fig. 1, the unsaturated bile acids (Δ^{7}, $\Delta^{8(14)}$ and Δ^{14}) (I—IV) give positive plain curves similar to that of cholic acid (XVIII). The background rotations of the above acids, therefore, are not affected by the introduction of these double bonds.

Some kinds of unsaturated keto bile acids with carbonyl groups at C_3 and/or C_{12} were prepared; by measuring their RD curves, the effect of the positions of the double bonds on the RD curves of the parent saturated keto bile acids was studied.

A) Unsaturated 3-Keto Bile Acids.—*A—1) Compounds with Double Bonds in Ring A.*—Methyl 3-keto-Δ^{4}-cholenate (V) has the same nuclear structure as Δ^{4}-cholestene-3-one, and, as expected, it gives a multiple negative Cotton-effect RD curve, similar to that of Δ^{4}-cholestene-3-one⁸⁾, as is shown in Fig. 2.

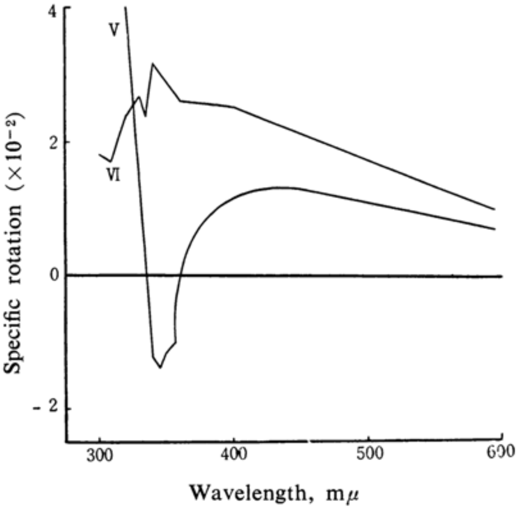

Fig. 2. Rotatory dispersion curves of: methyl 3-keto-Δ^{4}-cholenate (V) and methyl 3-keto-$\Delta^{7,9(11)}$-choladienate (VI).

A—2) Compounds with Isolated Double Bonds in Ring C or D.—The unsaturated bile acids VII—X show negative Cotton-effect curves which are not only similar to one another, but also resemble that of methyl 3-keto-12-acetoxycholanate (XIX) (Fig. 3). The result clearly indicates that the introduction of double bonds at positions remote from the carbonyl groups of these bile acids does not exert remarkable effects on the RD curves, though the intensity of the trough in the curves is more or less increased.

A—3) Compounds with Double Bonds in Ring B.—Figure 4 illustrates the RD curves of methyl 3-keto-Δ^{7}-cholenate (XI) and methyl 3-keto-Δ^{6}-cholenate (XII). Though the RD curve of

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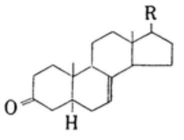
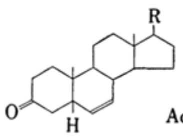
9) C. Djerassi, "Optical Rotatory Dispersion", McGraw-Hill Book Co., Inc., New York (1960), p. 63.


XI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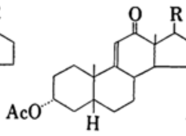
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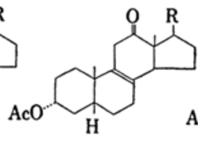
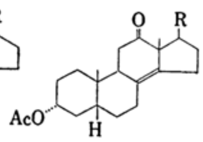
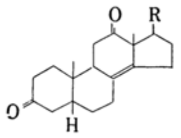
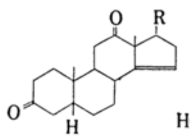

XIV


XV


XVI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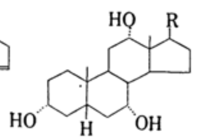
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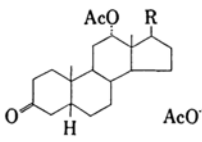
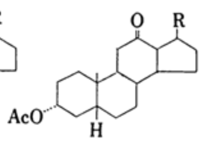
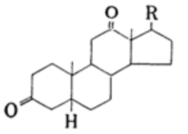

XIX


XX


XXI

R = $-CH(CH_3)CH_2CH_2COOCH_3$

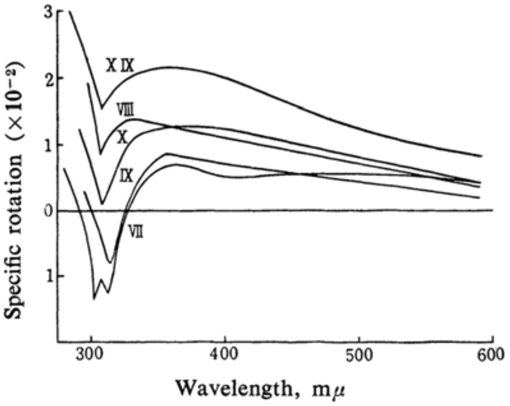


Fig. 3. Rotatory dispersion curves of: methyl 3-keto-Δ^8-cholenate (VII), methyl 3-keto-$\Delta^{8(14)}$-cholenate (VIII), methyl 3-keto-$\Delta^{9(11)}$-cholenate (IX), methyl 3-keto-Δ^{14}-cholenate (X) and methyl 3-keto-12-acetoxycholanate (XIX).

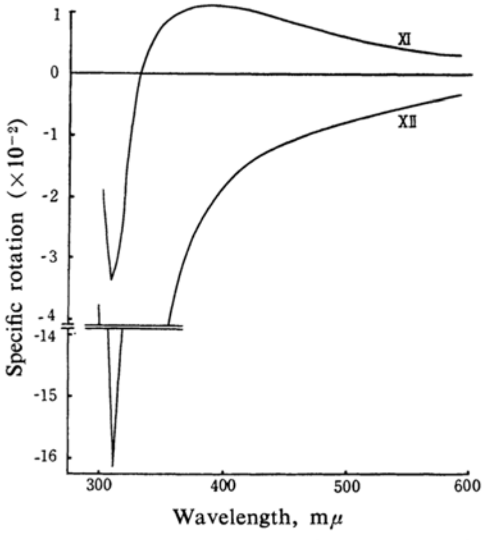


Fig. 4. Rotatory dispersion curves of: methyl 3-keto-Δ^7-cholenate (XI) and methyl 3-keto-Δ^6-cholenate (XII).

the former XI shows a negative drift greater than those of the above-mentioned compounds with double bonds in ring C or D (A—2), it must be noted that all of these curves are of the same shape. The RD curve of the Δ^6-compound XII is likewise a negative Cotton-effect curve of the same type and, indeed, of a strikingly high intensity (α_{312} $-1615°$). Since the RD curve of the 3-keto-steroid of the 5α-series with an isolated double bond in ring B hardly differs from that of the corresponding saturated ketone, as Djerassi⁹⁾ has pointed out, such a characteristic phenomenon as is given by the above Δ^6-compound XII might be attributable to the cis A/B fusion of this compound and will serve for the diagnosis of the position of the double bond in the unsaturated 3-keto-5β-steroids.

Djerassi et al.¹⁰⁾ reported that the introduction of a second double bond ($\Delta^{9(11)}$) in Δ^7-lanostene-3-one results in a strong positive

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10) C. Djerassi and O. Halpern, *J. Am. Chem. Soc.*, **80**, 4001 (1958).

shift of the RD curve. Methyl 3-keto-$\Delta^{7,9(11)}$-choladienate (Fig. 2, VI), which has a structure similar to the lanostadiene derivative, likewise shows a positive, but irregularly shaped, shift of the RD curve of the Δ^{7}-bile acid (XI).

B) Unsaturated 12-Keto Bile Acids. — As reported by Djerassi et al.[8], a series of saturated 12-keto bile acids give positive Cotton-effect curves with peaks on the positive side. The effect of the introduction of double bonds in ring C or D on the RD curves of some 12-keto bile acids was found to be as follows:

B—1) Compounds with α,β-Unsaturated 12-Keto Groups.—The RD curve of methyl 3α-acetoxy-12-keto-$\Delta^{9(11)}$-cholenate (XIII) (Fig. 5) shows a multiple negative Cotton-effect curve quite similar in shape to those of cholestene-3-one and methyl 3-keto-Δ^{4}-cholenate (V) mentioned above (A—1); i. e., the introduction of a double bond conjugated to the keto group resulted in the strong negative shift of the RD curve displayed by the saturated 12-keto bile acid[8]. This phenomenon can be well explained by the fact that the B/C bicyclic system including the $\Delta^{9(11)}$-12-ketone group XIII has quite the same configuration as the A/B bicyclic system with the Δ^{4}-3-ketone group V.

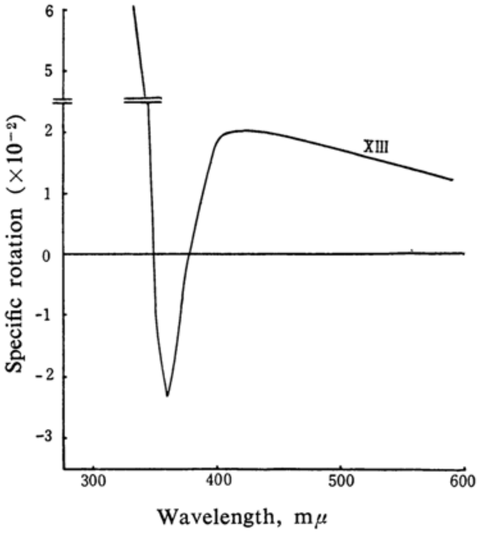

Fig. 5. Rotatory dispersion curves of methyl 3α-acetoxy-12-keto-$\Delta^{9(11)}$-cholenate (XIII).

B—2) Compounds with Isolated Double Bonds in Ring C.—It is very interesting to note that, while the RD curve of methyl 3α-acetoxy-Δ^{8}-cholenate (XIV) gives a positive Cotton-effect curve similar to that of the saturated 12-keto-cholanic acid derivative XX, methyl 3α-acetoxy-12-keto-$\Delta^{8(14)}$-cholenate (XV) shows a very strong negative shift of the RD curve, as is illustrated in Fig. 6. Since methyl 3α-acetoxy-$\Delta^{8(14)}$-cholenate (III) shows a positive background rotation just like the saturated bile acids (Fig. 1), such an inversion of the Cotton-effect as is shown by the $\Delta^{8(14)}$-12-keto bile acid XV might be attributed to a possible fact that the environment surrounding the C_{12}-carbonyl group of the bile acid XX is altered by the introduction of the $\Delta^{8(14)}$-bond so as to result in a strong inversion of the Cotton-effect.

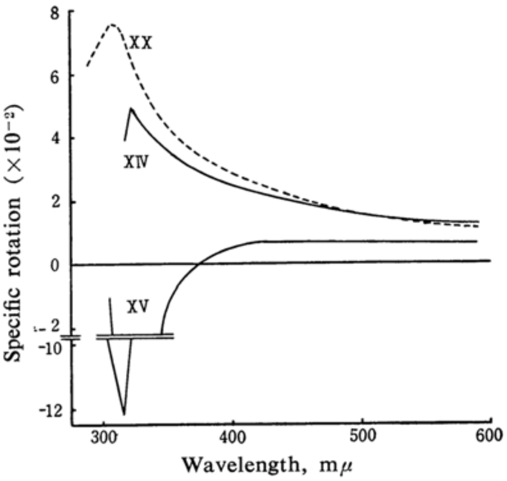

Fig. 6. Rotatory dispersion curves of: methyl 3α-acetoxy-12-keto-Δ^{8}-cholenate (XIV), methyl 3α-acetoxy-12-keto-$\Delta^{8(14)}$-cholenate (XV) and methyl 3α-acetoxy-12-keto-cholanate (XX).

B—3) 3,12-Diketo Bile Acids with Double Bonds in Ring C or D.—Previously Djerassi et al.[8]

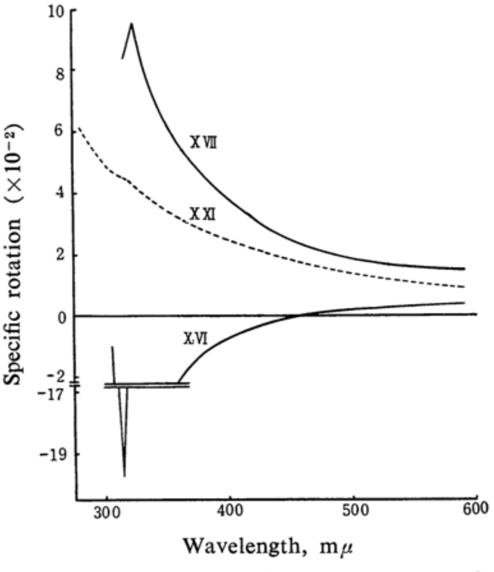

Fig. 7. Rotatory dispersion curves of: methyl 3, 12-dikoto-$\Delta^{8(14)}$-cholenate (XVI), methyl 3, 12-diketo-Δ^{14}-cholenate (XVII) and methyl 3, 12-diketocholanate (XXI).

reported that the RD curves of saturated diketo bile acids, such as 3, 12-diketocholanic acid (XXI), can be calculated by superimposing the RD curve of 3-ketocholanic acid on that of 12-ketocholanic acid. As samples of unsaturated diketo bile acid, methyl 3, 12-diketo-$\Delta^{8(14)}$-cholenate (XVI) and methyl 3, 12-diketo-Δ^{14}-cholenate (XVII) were prepared and their RD curves were determined. The curves are shown in Fig. 7. Such an inversion of the Cotton-effect as is shown above (B—2) is also observed in the RD curve of the $\Delta^{8(14)}$-diketo bile acid (XVI), and the negative Cotton-effect given by methyl 3α-acetoxy-12-keto-$\Delta^{8(14)}$-cholenate (XV) is increased by the introduction of the 3-keto group. The RD curve of the Δ^{14}-diketo bile acid (XVII) shows that the positive Cotton-effect RD curve of the saturated 12-ketocholanic acid XX is superimposed by the curve of methyl 3-keto-Δ^{14}-cholenate (X).

Experimental*

Methyl 3α-Hydroxy-Δ^7-cholenate1) (I).—M. p. 108°C, $[\alpha]_D^{24}$ +61° (chf.). IR ν_{max}^{Nujol}; 846 cm^{-1} (=C-H), 1738 cm^{-1} (C=O), 3366 cm^{-1} (OH). RD (Fig. 1); $[\alpha]_{589}^{19.5}$ +85°, $[\alpha]_{400}$ +256°, $[\alpha]_{320}$ +416° (Zr).

Methyl 3α-Acetoxy-Δ^7-cholenate1) (II).—M. p. 125°C, $[\alpha]_D^{23}$ +86° (chf.). IR ν_{max}^{Nujol}; 808 cm^{-1} (=C-H), 1241 cm^{-1} (C-O-C), 1745 cm^{-1} (C=O), 1678 cm^{-1} (C=C); RD (Fig. 1); $[\alpha]_{589}^{19.5}$ +45°, $[\alpha]_{400}$ +188°, $[\alpha]_{320}$+322° (Zr).

Methyl 3α-Acetoxy-$\Delta^{8(14)}$-cholenate2) (III).—M. p. 81～83°C, $[\alpha]_D^{18}$ +52° (chf.). IR ν_{max}^{Nujol}; 1200 cm^{-1} (C-O-C), 1740 cm^{-1} (C=O). RD (Fig. 1); $[\alpha]_{589}^{19.5}$ +50°, $[\alpha]_{400}$ +168°, $[\alpha]_{320}$ +286° (Zr).

Methyl 3α-Hydroxy-Δ^{14}-cholenate (IV)1,2).—M. p. 141°C, $[\alpha]_D^{20}$ +55° (chf.). IR ν_{max}^{Nujol}; 793 cm^{-1} (=C-H), 1743 cm^{-1} (C=O), 3299 cm^{-1} (OH). RD (Fig. 1); $[\alpha]_{589}^{19.5}$ +47°, $[\alpha]_{400}$ +116°, $[\alpha]_{320}$ +164° (Zr).

Methyl 3-Keto-Δ^4-cholenate11) (V).—M. p. 125°C, $[\alpha]_D^{21}$ +61° (chf.). IR ν_{max}^{Nujol}; 800 cm^{-1} (=C-H), 1680 cm^{-1} (C=C), 1740 cm^{-1} (C=O). RD (Fig. 2); $[\alpha]_{589}^{23.3}$ +70°, $[\alpha]_{400}$ +117°, $[\alpha]_{350}$ −120°, $[\alpha]_{345}$ −138° (broad trough), $[\alpha]_{340}$ −125° (Zr).

Found: C, 77.43; H, 10.04. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.67; H, 9.91%.

Methyl 3-Keto-$\Delta^{7,9(11)}$-choladienate12) (VI).—M. p. 144°C, $[\alpha]_D^{21}$ +88° (chf.). IR ν_{max}^{Nujol}; 800 cm^{-1} (=C-H), 1709 cm^{-1}, 1738 cm^{-1} (C=O). RD (Fig. 2); $[\alpha]_{589}^{26.7}$ +100°, $[\alpha]_{400}$ +250°, $[\alpha]_{340}$ +315° (peak), $[\alpha]_{335}$ +227° (trough), $[\alpha]_{330}$ +268° (peak), $[\alpha]_{325}$ +259° (Zr).

Found: C, 78.06; H, 9.36. Calcd. for $C_{25}H_{36}O_3$ (384.54): C, 78.08; H, 9.44%.

Methyl 3-Keto-Δ^8-cholenate (VII).—3α-Acetoxy-12-keto-Δ^8-cholenic acid (m. p. 148°C) (200 mg.), synthesized by the method reported by Fieser et al.13), was reduced by the Huang-Minlon procedure (hydrazine hydrate, 5 ml. and potassium hydroxide, 5 g.). The resulting free acid (m. p. 173°C) was methylated with diazomethane, and the product was oxidized according to the method of Bladon et al.3) The oxidation product was chromatographed and recrystallized from methanol. Yield, 68 mg., m.p. 122°C, $[\alpha]_D^{20}$ +22° (chf.). IR ν_{max}^{Nujol}; 1715 cm^{-1}, 1740 cm^{-1} (C=O). RD (Fig. 3); $[\alpha]_{589}^{28.3}$ +18°, $[\alpha]_{400}$ +66°, $[\alpha]_{310}$ −125°, $[\alpha]_{302.5}$ −134° (trough), $[\alpha]_{300}$ −73° (Xe).

Found: C, 77.50; H, 9.84. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.65; H, 9.91%.

Methyl 3-Keto-$\Delta^{8(14)}$-cholenate (VIII).—3α-Hydroxy-$\Delta^{8(14)}$-cholenic acid (m. p. 168°C) (100 mg.), derived from apocholic acid2), was methylated with diazomethane, and the ester was oxidized according to the method of Bladon et al.3) Yield, 56 mg., m. p. 104°C, $[\alpha]_D^{19}$ +68° (chf.). IR ν_{max}^{Nujol}; 1710 cm^{-1}, 1730 cm^{-1} (C=O). RD (Fig. 3); $[\alpha]_{589}^{16.4}$ +39°, $[\alpha]_{400}$ +112°, $[\alpha]_{310}$ +105°, $[\alpha]_{307.5}$ +87° (trough), $[\alpha]_{305}$ +115° (Zr.)

Found: C, 77.19; H, 9.80. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.65; H, 9.91%.

Methyl 3-Keto-$\Delta^{9(11)}$-cholenate (IX).—Methyl 3α-acetoxy-12-keto-$\Delta^{9(11)}$-cholenate (200 mg.) obtained by the method reported by Fieser et al.13), was reduced by the Huang-Minlon method as mentioned above, and the product (its methyl ester) was purified by chromatography. Methyl 3α-hydroxy-$\Delta^{9(11)}$-cholenate (m. p. 103°C) thus obtained was oxidized with a chromic acid-sulfuric acid mixture3). The oxidation product was recrystallized from methanol. M. p. 106°C, $[\alpha]_D^{21}$ +33° (chf.). IR ν_{max}^{Nujol}; 825 cm^{-1} (=C-H), 1720 cm^{-1}, 1738 cm^{-1} (C=O); RD (Fig. 3): $[\alpha]_{589}^{21}$ +41°, $[\alpha]_{400}$ +102°, $[\alpha]_{317.5}$ −66°, $[\alpha]_{315}$ −81° (broad trough), $[\alpha]_{312.5}$ −76°.

Found: C, 77.01; H, 9.83. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.67; H, 9.91%.

Methyl 3-Keto-Δ^{14}-cholenate (X)1).—M. p. 89°C, $[\alpha]_D^{24}$ +38° (chf.). IR ν_{max}^{Nujol}; 777 cm^{-1} (=C-H), 1715 cm^{-1}, 1742 cm^{-1} (C=O). RD (Fig. 3); $[\alpha]_{589}^{19}$ +42°, $[\alpha]_{400}$ +122°, $[\alpha]_{310}$ +14°, $[\alpha]_{307.5}$ +10° (trough), $[\alpha]_{305}$ +24 (Zr).

Methyl 3-Keto-Δ^7-cholenate1) (XI).—M. p. 125°C, $[\alpha]_D^{23}$ +62° (chf.). IR ν_{max}^{Nujol}; 795 cm^{-1} (=C-H), 1712 cm^{-1}, 1740 cm^{-1} (C=O). RD (Fig. 4); $[\alpha]_{589}^{20.8}$ +29°, $[\alpha]_{400}$ +110°, $[\alpha]_{310}$ −322°, $[\alpha]_{307.5}$ −334° (trough), $[\alpha]_{305}$ −186° (Zr).

Methyl-3-Keto-Δ^6-cholenate (XII).—3α-Hydroxy-Δ^6-cholenic acid (m. p. 214°C) prepared according to the method of Schubert et al.14) was methylated with diazomethane, and the product was oxidized with a chromic acid-sulfuric acid mixture3). M. p. 125°C, $[\alpha]_D^{22}$ −52° (chf.). IR ν_{max}^{Nujol}: 740 cm^{-1} (=C-H), 1717 cm^{-1}, 1743 cm^{-1} (C=O). RD (Fig. 4);

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* Ultraviolet absorption spectra were measured in ethanol solutions with a Beckman spectrophoto-densitometer (Model DU); rotatory dispersion, in methanol solutions (concentration: 0.1%), with a Rudolph high precision photoelectric polarimeter (Model 200S-80Q) (light source: zirconium lamp (Zr) or xenon arclamp (Xe)).

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$[\alpha]_{589}^{28.2}$ −33°, $[\alpha]_{400}$ −178°, $[\alpha]_{315}$ −1524°, $[\alpha]_{312.5}$ −1615° (trough), $[\alpha]_{310}$ −1485° (Xe and Zr).

Found: C, 77.52; H, 9.85. Calcd. for $C_{25}H_{38}O_3$ (386.55): C, 77.67; H, 9.91%.

Methyl 3α-Acetoxy-12-keto-$\Delta^{9(11)}$-cholenate (XIII). — 3α-Hydroxy-12-keto-$\Delta^{9(11)}$-cholenic acid (m. p. 117°C), obtained by Fieser's method13), was methylated (diazomethane) and then acetylated as usual. M. p. 144°C, $[\alpha]_D^{22}$+110° (chf.). IR ν_{max}^{Nujol}; 1245 cm⁻¹ (C-O-C), 1680 cm⁻¹, 1730 cm⁻¹, 1740 cm⁻¹ (C=O). RD (Fig. 5); $[\alpha]_{589}^{22.1}$ +128°, $[\alpha]_{400}$ +185°, $[\alpha]_{365}$ −203°, $[\alpha]_{360}$ −236° (trough), $[\alpha]_{357.5}$ −184° (Xe).

Found: C, 73.06; H, 901 Calcd. for $C_{27}H_{40}O_5$ (444.617): C, 72.94; H, 9.07%.

Methyl 3α-Acetoxy-12-keto-Δ^8-cholenate (XIV). —This compound was prepared by the method reported by Fieser et al.13) M. p. 131°C, $[\alpha]_D^{22}$ +138° (chf.). IR ν_{max}^{Nujol}; 120 cm⁻¹, 1255 cm⁻¹ (C-O-C), 1705 cm⁻¹, 1734 cm⁻¹ (C=O); RD (Fig. 6); $[\alpha]_{589}^{28}$ +123°, $[\alpha]_{400}$ +263°, $[\alpha]_{325}$ +468, $[\alpha]_{322.5}$ +491° (peak), $[\alpha]_{320}$ +455°.

Found: C, 72.97; H, 8.99. Calcd. for $C_{27}H_{40}O_5$ (444.59): C, 72.94; H, 9.07%.

Methyl 3α-Acetoxy-12-keto-$\Delta^{8(14)}$-cholenate (XV). —3α-Ethoxycarbonyloxy-12-keto-$\Delta^{8(14)}$-cholenate (m. p. 116°C), prepared by the method reported previously2), was hydrolyzed by boiling over a water bath with 20 ml. of a 3% methanolic potassium hydroxide solution. The free acid obtained was methylated (diazomethane) and then acetylated as usual. M. p. 98°C, $[\alpha]_D^{19}$ +55° (chf.). IR ν_{max}^{Nujol}; 1245 cm⁻¹ (C-O-C), 1720 cm⁻¹, 1730 cm⁻¹ (C=O); RD (Fig. 6); $[\alpha]_{589}^{22.5}$ +57°, $[\alpha]_{400}$ +52°, $[\alpha]_{317.5}$ −1188°, $[\alpha]_{315}$ −1228° (trough), $[\alpha]_{312.5}$ −1169° (Xe).

Found: C, 73.12; H, 9.08. Calcd. for $C_{27}H_{40}O_5$ (444.59): C, 72.94; H, 9.07%.

Methyl 3, 12-Diketo-$\Delta^{8(14)}$-cholenate (XVI).— Methyl apocholate (m. p. 82°C) was oxidized with a chromic acid-sulfuric acid mixture3). M. p. 120°C, $[\alpha]_D^{18}$ +14° (chf.). IR ν_{max}^{Nujol}; 1740 cm⁻¹, 1715 cm⁻¹ (C=O). RD (Fig. 7); $[\alpha]_{589}^{28.2}$ +32°, $[\alpha]_{400}$ −67°, $[\alpha]_{320}$ −1384°, $[\alpha]_{317.5}$ −1986° (trough), $[\alpha]_{315}$ −1455° (Xe).

Found: C, 74.59; H. 8.99. Calcd. for $C_{25}H_{36}O_4$ (346.56): C, 74.96; H, 9.06%.

Methyl 3, 12-Diketo-Δ^{14}-cholenate1).—M. p. 134°C, $[\alpha]_D^{22}$ +60° (chf.). IR ν_{max}^{Nujol}; 780 cm⁻¹ (=C-H), 1705 cm⁻¹, 1738 cm⁻¹ (C=O). RD (Fig. 7); $[\alpha]_{589}^{20}$ +141°, $[\alpha]_{400}$ +380°, $[\alpha]_{322.5}$ +955° (peak), $[\alpha]_{320}$ +916° (Xe).

Summary

Not only ultraviolet and infrared absorption spectra but also optical rotatory dispersions of various unsaturated bile acids were studied, and most of the data were found to be in good agreement with those hitherto obtained concerning the other steroids. The determination of the rotatory dispersion gave the following results:

1) The background rotation of the saturated bile acid is not affected by the introduction of a double bond in ring B, C or D.

2) The introduction of a double bond in ring C or D does not remarkably affect the RD curve of the saturated 3-keto bile acids, while the negative drift of the RD curve given by 3-keto-Δ^6-cholenic acid is so very striking that this phenomenon could be utilized for the diagnosis of Δ^6-3-keto bile acids (5β).

3) The positive Cotton-effect RD curve given by a saturated 12-keto bile acid is strongly inverted by the introduction of the $\Delta^{8(14)}$-bond, but not by that of the Δ^8-bond.

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